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Plasmid DNA Hydrogels for biomedical applications

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Abstract

In the last few years, our research group has focused on the design and development of plasmid DNA (pDNA) based systems as devices to be used therapeutically in the biomedical field. Biocompatible macro and micro plasmid DNA gels were prepared by a cross-linking reaction. For the first time, the pDNA gels have been investigated with respect to their swelling in aqueous solution containing different additives. Furthermore, we clarified the fundamental and basic aspects of the solute release mechanism from pDNA hydrogels and the significance of this information is enormous as a basic tool for the formulation of pDNA carriers for drug/gene delivery applications. The co-delivery of a specific gene and anticancer drugs, combining chemical and gene therapies in the treatment of cancer was the main challenge of our research. Significant progresses have been made with a new p53 encoding pDNA microgel that is suitable for the loading and release of pDNA and doxorubicin. This represents a strong valuable finding in the strategic development of systems to improve cancer cure through the synergetic effect of chemical and gene therapy.

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Introduction

The evolution in the design and preparation of hydrogels, namely at the micro and nanoscale, has a great deal of interest in biomaterials science because of their tunable chemical and physical three-dimensional structure, good mechanical properties, high water content and biocompatibility to tissue and blood.[1-6] These characteristics give potential value for the use of hydrogels in biomedical implants, tissue engineering, bionanotechnology and drug/gene delivery.[7-11] Concerning this last issue, a major research thrust in the biochemical/pharmaceutical technology is still the development of efficient and safe controlled release systems for the sustained delivery of drugs and bioactive agents. To be used therapeutically, these systems should be able to deliver the drug and/or gene at a specified rate and time period ensuring that the concentration of the biopharmaceutical in the body would be kept in the desirable range for a prolonged time. Additionally, the carrier can be targeted to a particular cell type or an organ;[12-14] not only protein drugs would benefit from a targeted delivery to their site of action, but also highly toxic ones such as anticancer drugs.[15-19] The creation of polymer networks tailoring intrinsic properties, such as, ionization of the gel, the extent of swelling and its reversibility, and specific mesh size appears as a valuable tool in engineering appropriate release devices. Development of temperature-responsive hydrogels,[20,21] pH-responsive networks[22] and glucose-responsive hydrogels[23,24] were conceived to improve the field of sustained/controlled drug delivery. In order to enhance their practical efficacy in the biomedical area, mathematical models can be applied to correlate material properties, interaction parameters, kinetic events, and transport behaviour within complex hydrogel systems.[25-27] Among the several physical/chemical properties of hydrogels that can be manipulated to ensure greater delivery performance, both the gel swelling and biodegradability are remarkable strategies. The swelling is restricted by the cross-linking density in the network and the deswelling by the volume occupied by the polymer network. There are several studies in the literature reporting the controlled release of drugs from biodegradable hydrogels,[28,29] such as the release of recombinant human interleukin-2 from dextran-based hydrogels;[30] similar dextran hydrogels were investigated as drug delivery devices for the posterior part of the eye.[31] Moreover, the polymer hyaluronic acid (HA) was used to create a combination tissue engineering scaffold and protein delivery vehicle.[32] Recently, in situ forming biodegradable poly(ethylene glycol) based hydrogels were created for the time-controlled release of

tethered peptides or proteins[33] and injectable biodegradable hydrogels were studied as vehicles for the release of anticancer drugs.[34] Biodegradable hydrogels appears to be quite suitable for DNA release, once one can readily control the release rate by modulating the network structure with adjusting cross-linking density.[35,36]

The field of gene therapy, with either viral or synthetic vectors, attracts great and widespread interest, due to its technological challenging characteristics and for providing great opportunities for treating diseases due to genetic disorders, infections and cancer.[37] The use of synthetic vectors is an incredible improvement and has many assets, such as, ease and variability of preparation; lack of immune response and unlimited DNA-carrying capacity.[38,39] Controlled gene release ensures the increase of the extent and duration of transgene expression, reduced need for multiple interventions and lower toxicity to non-target cells.[40-47] Applied to cancer treatment, gene therapy purposes using hydrogel devices bring impressive progresses feeding the hope of cancer cure.[48-52] Several interesting studies reporting the efficiency of p53 gene cancer therapy are available in the literature.[53-58] In line with this, and in an attempt to evolve in the cancer cure, a new strategy seems to be the combination of chemical and gene therapy improving the treatment efficacy due to their synergistic effect. Simultaneously with anticancer drugs, specific genes can be delivered to the same cancer cells to enhance sensitivity of targeted cells to drug, during all the treatment. Over the past decade, a few studies reporting the delivery of nucleic acids and drugs have been presented,[59-63] illustrating the advantage of its combined effect in medical care. This bifunctionality may represent an enormous advance in comparison to individual chemotherapy treatments and will, certainly, serve as a basis to improve cancer therapies. Recently, a new p53 encoding plasmid DNA (pDNA) microgel that is porous, biocompatible, and photodegradable, thus suitable for the loading and release of pDNA and, e.g., doxorubicin, an intrinsically fluorescent anticancer drug widely used in cancer treatment, has been developed.[64]

This review represents our contribution to the field of biomaterials design, and concerns the synthesis of new plasmid DNA vehicles with potential biomedical application. The interesting features found in the proposed systems ranged from responsive swelling properties, light triggered release profile and promising drug/gene co-delivery approach in cancer therapeutics.

Plasmid DNA gels formation and characterization

In the last decade, we assisted to a growing knowledge in the scientific area dealing with delivery systems of nucleic acids. In an attempt to better understand DNA trafficking and expression, new strategies based on liposomal[65-73] and polymeric[74-85] formulations emerged for the efficient delivery of theses vectors toward cells. Our group focused on the synthesis of chemical pDNA based hydrogels by cross-linking reaction with ethylene glycol diglycidyl ether (EGDE). The reaction mechanism of gel formation seems to involve the guanine nitrogen atom at position seven (N-7), which attacks the more substituted carbon of the epoxide or the least hindered end of the epoxide. The reaction is of nucleophilic substitution type (S_N2 reaction). All pDNA gels are clear and transparent (Figure 1). The knowledge of the equilibrium degree of swelling allows for the calculation of matrix structural parameters such as the distance between cross-links and the mesh size of the gel. Gels with different cross-linker densities were prepared.[86] Following the method of determination described by Canal and Peppas,[87] the mesh size has been determined.[88] Information about the integrity of double helix of the DNA molecules in the gels was obtained by fluorescence microscopy using acridine orange (AO), as a dye.[89,90] AO intercalates into double stranded DNA as a monomer, whereas it binds to the single stranded DNA conformational state as an aggregate. Upon excitation at 470 nm to 490 nm, the acridine orange monomeric form binds to ds-DNA and fluoresces green. The aggregated acridine orange on ss-DNA fluoresces red.[91] Through the observation of green or red fluorescence, DNA fluorescence microscopy studies with AO have revealed the DNA conformational state in the gels. Figure 2 shows fluorescence micrograph (A) and scanning electron micrograph (B) of a plasmid DNA gel, respectively. Both images demonstrated that the gels show a porous and smooth surface. Fluorescence microscopy studies have also revealed that the formation of the gels was carried out with the conservation of plasmid DNA double helix integrity. Complementary, scanning electron micrographs allow to conclude that those gels show a three-dimensional coral-like spongy structure with small cavities confined by perforated membranes. Moreover, cell viability assays suggested that the pDNA gels are non-toxic to cells and this step contributes to the possibility of using them as carriers in real biological systems. As expected, toxicity increases with cross-linking density.[86]

Swelling behaviour of Plasmid DNA gels

In pure water, charged networks swell due to the osmotic pressure from the counterions, which originates from their translational entropy. The driving force of the swelling process is the presence of mobile osmotically active counterions. The volume changes of gels are associated with osmotic effects and not dehydration.[92-95] The extent of the collapse is affected by molecular parameters of the network such as the cross-linking density, the hydrophobic/hydrophilic balance of the polymer network, its content of charged groups, its flexibility and its ability to interact with added solutes. In neat water, the degree of swelling for 0.2% EGDE plasmid DNA gels is considerably larger than that of corresponding 0.5% EGDE gels. A lower cross-linker density leads, as expected, to an increased swelling. An increase in the level of cross-linking agent leads to a larger resistance to the osmotic swelling force and thus to a reduced equilibrium swelling.[86] Furthermore, the swelling behaviour of covalently cross-linked pDNA on addition of different cosolutes, which include inorganic salts with different cation valency, polyamines such as spermine and spermidine, cationic macromolecules such as lysozyme and chitosan, and different classes of surfactants was studied.[86] On addition of a monovalent electrolyte, there is a progressive contraction of the gels. When gels, preswollen in the 1 mM NaOH, are placed into salt solutions at different concentrations, they shrink due to the screening effect of the salt and mainly due to the concentration difference of mobile ions inside the gel and the external solution governed by the Donnan equilibrium. In the presence of salt, the difference between ion concentrations inside and outside the gel is reduced. Consequently, the driving force of swelling decreases gradually with increasing salt concentration. In addition, Pourjavadi et al. recognized the lower absorbent capability of hydrogels in the presence of salts and proposed a parameter to measure the salt effect on the water absorbency capacity by correlating the hydrogel swelling in pure water and that in a salt aqueous solution.[96]

The nature of the monovalent counterion has only a moderate effect on the deswelling of plasmid DNA gels. The degree of collapse follows the order $Na^+ > K^+ > Rb^+ > Cs^+$, with the sodium ion showing the highest ability in collapsing pDNA gels.[86] The order observed suggests that monovalent cations with a smaller ionic radius are more efficient in the collapse of pDNA gels. It is known that monovalent alkali cations are involved in the regulation of important biological processes and also influence the DNA compaction

in living cells. Compaction of DNA by monovalent cations alone does not occur but can be promoted by an increase in the concentration of monovalent salts.[97] Studies of the influence of the cation nature on the ability to induce DNA compaction in solution have been performed.[98] The deswelling on addition of the divalent salts $CaCl_2$, $MgCl_2$ and $SrCl_2$ occurs at considerably lower salt concentrations and the deswelling appears to be more pronounced, as compared with the monovalent metal ions. The collapse ratios for these gels show a trend in the order $CaCl_2 > MgCl_2 > SrCl_2$, with calcium being the most effective ion in collapsing plasmid DNA gels.[86] Concerning the poly-cationic species chitosan, spermine (Spm), spermidine (Spd) and lysozyme, it was found that chitosan is the most efficient co-solute in promoting the gel collapse, followed by the polyamines and finally lysozyme. The different charges carried by these cationic species confer them characteristic swelling behaviour. The higher charge of spermine compared to spermidine evidently makes spermine bind more strongly to pDNA with a concomitant higher gel collapse potential. A considerably larger concentration of lysozyme was needed to reach a corresponding collapse of the gel, than for the other polycationic species. Lysozyme has a rather low density of surface charges, since the cationic groups are located among uncharged and anionic amino-acid residues on the protein surface. A contribution to the difference can also comes out from steric effects, i.e., a linear polymer penetrates more easily into the network than a globular one.

In the bulk phase and at low concentrations, the surfactant molecules are dissolved as unimers, whereas at higher surfactant concentrations a self-assembly into aggregates occurs. For the single-chain surfactants, the aggregates formed in this self-assembly are commonly spherical micelles with micelle formation starting at a well-defined concentration, the critical micelle concentration (cmc).[99] In the presence of an oppositely charged polyelectrolyte, the micelle formation of an ionic surfactant is strongly facilitated leading to a major lowering of the cmc.[100] The stabilisation of micelles due to an oppositely charged polyelectrolyte is mainly an entropic effect, due to a release of counterions.[101-105] As expected, the cationic surfactant binding is particularly strong for anionic polyelectrolytes of high charge density.[101] The plasmid DNA gels are highly swollen due to the osmotic pressure arising from the counterions, which are confined to the gel. After the immersion of the swollen pDNA gels in the solutions of the oppositely charged surfactants, the surfactant ions migrate into the network and replace the network counterions, which are released. Adsorption of a

considerable amount of C_nTA^+ ions leads to a transition of the swollen network to the collapsed state. The main reason for this transition is thus the aggregation of surfactant ions within the pDNA gel due to hydrophobic interactions between their hydrocarbon chains. As a consequence of this, the mobile counterion concentration in the network decreases, leading to a significant decrease in the internal osmotic pressure in the gel. Furthermore, the surfactant aggregates will act as multivalent counterions and by ion correlation effects contribute to the contraction of the gel. Figure 3 shows swelling isotherms for plasmid DNA gels on addition of a number of cationic surfactants. The surfactants have no effect at lower concentrations but there is a marked deswelling at higher concentrations, which becomes more important the longer the surfactant alkyl chain. The concentration of onset of deswelling varies by orders of magnitude between different surfactants; additionally, the plateau value obtained at high surfactant concentrations is lower by increasing the alkyl chain length.[86] The pronounced chain length dependences directly suggest a dominant role of surfactant self-assembly. These results for the different alkyl chain lengths confirm that the deswelling occurs below the normal critical micelle concentration of the surfactant. The cmc values for C_{16}TAB, C_{14}TAB, C_{12}TAB and C_8TAB are 0.9 mM, 0.23 mM, 15 mM and 144 mM, respectively. We found that the surfactants induce the volume transition starting at a certain rather well-defined concentration, critical association concentration, cac ~ 0.015 mM for C_{16}TAB, cac ~ 0.045 mM for C_{14}TAB, cac ~ 0.08 mM for C_{12}TAB and cac ~ 1 mM for C_8TAB.[120] Furthermore, the swelling of the gels appears to be reversible, as exemplified by the deswelling/swelling process induced by consecutive addition of cationic and anionic surfactant. The relative V/V_0 returned to between 90 and 100% of the initial state. The interaction between the two surfactants is stronger than that between a cationic surfactant and plasmid DNA. The dynamic deswelling-swelling process could be useful in the control of the release rate of solutes from gels via "on-off" switching.

Light triggered release from Plasmid DNA gels

Our expertise on the gel swelling behaviour, compaction of DNA by cationic entities, as lipids and surfactants as well as on the assembly structures of these complexes allow us for the development of novel systems to be used in a variety of biomedical

applications.[36,86,106-113] Once the physicochemical characterization of pDNA hydrogels has been established,[86] we evolved to the challenge of using pDNA-based carriers in the biological area. During the research of pDNA gel as delivery system it was found that the pDNA network is photodegradable.[88] The first evidence of gel degradation comes from the pDNA release after gels being irradiated with light. After irradiation, both gels with different degrees of crosslinking (0.2 % and 0.5 % ethylene glycol diglycidyl ether (EGDE)) suffered disruption leading to the release of plasmid DNA with time (Figure 4). The release is characterized by a narrow time lag in the first 24 hours, after which the release gradually increases until a plateau is reached around 400 hours of photodegradation. The initial time lag, 18 and 23 hours for 0.2 % and 0.5 % EGDE gels respectively, may be related to the number of cross-links that have to be degraded to permit the release of plasmid DNA. After irradiation, and at maximum release, pDNA gels cross-linked with 0.2 % EGDE released 87.8 % of plasmid DNA while gels prepared from 0.5 % EGDE released 74.7 % of pDNA, in approximately 18 days. In the absence of ultraviolet light irradiation, and for both gel types, minimal amounts of pDNA, less than 8 %, are released. The degradation on ultraviolet light exposure (photo-oxidation) leads to the removal of the chemical cross-links and can allow the release of the constituent network polymer inducing changes in gel weight, mechanical properties, mesh size, porosity, and in the degree of swelling.[88]

In degradable gels, if the release of solutes is governed by polymer degradation, the degradation rate of the hydrogel must be matched to the size of the solute species. Moreover, control of variations with mesh size in time is crucial to design appropriate solute release devices. During hydrogel degradation, the water content tends to increase, increasing both the network mesh size and the volume swelling ratio and, consequently, the release of gel entrapped solutes is facilitated. To evaluate the dependence of the release rate on the size of an entrapped solute, lysozyme, BSA and FITC-dextran with molecular weights ranging from 14 100 to 77 000 Da, and the hydrodynamic radius being 16, 34.8 and 55 Å, respectively, were incorporated into 0.2 % and 0.5 % EGDE cross-linked pDNA gels.[88] The effect of radiation, on the release profiles of these molecules is presented in Figure 5. From the release curves it is possible to infer that approximately 98.6 % of lysozyme, 94.9 % of BSA and 96.6 % of FITC-dextran are released in 48, 192 and 274 hours, respectively, from 0.2 % EGDE pDNA gels. Similarly, approximately 94.9 % of lysozyme, 93.1 % of BSA and 94.1 % of FITC-

dextran are released in 72, 240 and 300 hours, respectively, from 0.5 % EGDE pDNA gels. In order to have a deep assessment on the release mechanism, the release kinetics has been fitted to Equations (1) and (2). Equation (1) is the simple power law Korsemeyer-Peppas equation[114]

$$C_t/C_\infty = kt^n \qquad (1)$$

where C_t and C_∞ are cumulative concentrations of the material released at time t and at infinite time, respectively, and k and n are fitting parameters, giving the later useful information on the release mechanism; for the release from cylinders (spheres/thin films), values of n near 0.45 (0.43/0.5) indicate a diffusion-controlled release (so-called Fickian). Non-Fickian behaviour is observed for $0.45 < n < 0.89$ ($0.43 < n < 0.85$ / $0.5 < n < 1.0$), with a limit of Case II (zero-order release) transports for $n = 0.89$. Non-Fickian and Case II transport are indicative of coupling of diffusional and relaxational mechanisms. Occasionally, values of $n > 0.89$ (0.85/1.0) has been observed and considered to be Super Case II kinetics.[114] An empirical approach, which allows describing the entire set of the release data, is based on the Weibull function

$$C_t = C_\infty[1-\exp(-k't)^d] \qquad (2)$$

where k' and d are constants. Papadopoulou et al.[115] have demonstrated that eq. (2) gives an insight into the diffusional mechanism, since the d and k' are closely related to the mechanism and rate constant of release, respectively. For BSA and FITC-Dextran n values are higher than 0.89 indicating a Super Case II release phenomena. This is also confirmed by d (Eq. 2) values higher than 1.[88] This mechanism emerges as the polymer resistance becomes more significant relative to the diffusion resistance; consequently, the release is linear at early times in the process, but at long time the release rate increases markedly. For the release of lysozyme, the analysis of the exponent d, suggests a Fickian release process. From the analysis of the release rate (k') of lysozyme, BSA and FITC-dextran from pDNA gels with different degrees of cross-linker we found that: a) k' decrease by increasing the degree of cross-linker and, b) for both gels, k' decreases from lysozyme to FITC-dextran by changing *ca.* two orders of magnitude.[88] Additionaly, for both cross-linker densities pDNA gels there is a correlation between the gel mesh size and the solute size. Not only drug size is

important but also the mobility of polymer chains and the extent of swelling are factors that should be taken into account.[88]

To further demonstrate the sustained release characteristics of the pDNA gel system, release studies were evaluated in vitro using selected anti-inflammatory drugs (hydrocortisone, cortisone, prednisolone, dexamethasone and prednisone). Modelling of the in vitro release profile is consistent with a Super Case II release mechanism, related with increases in the gel swelling degree. Furthermore, the release of the anti-inflammatory drugs depends on the balance between hydrodynamic volume and hydrophobicity and there is a correlation between drug release rate constant (k') and the hydrophobicity as quantified by the octanol-water partition coefficient; a slow release rate was found for the more hydrophobic drugs.[88] This shows that this biomaterial can be quite useful as a controlled release device for several therapeutic agents at wound sites.

Other authors took advantage of the photodegradable property to develop drug controlled release systems as valuable technology to be applied in the biomedical field. Han´s group designed photodegradable block copolymer micelles as nanocarrier for light-triggered release of guest molecules, which offers the remote-control possibility and enhances selectivity.[116] Other strategy with diblock copolymers has been used to promote in vitro photo-controlled drug delivery.[117] Fairbanks et al. synthesized hydrogels for the delivery of therapeutic agents as well as the study and manipulation of biological processes and tissue development. These gels are photodegradable and photoadaptable.[118] In a study using polyurethane based nanoparticles, it was demonstrated the promising way to trigger drug release in cells.[119] Considering that a deep penetration of nanosystems in tumors is required to increase its therapeutic value Tong and co-workers reported a novel photoswitchable carrier that provides spatiotemporal control of drug release and enhanced tissue penetration.[120] In the same issue, photodegradable macromers and hydrogels allow for live cell encapsulation and release.[121] Furtehrmore, for the first time the synthesis of photoresponsive polypeptide-based block copolymers has been established adding new possibilities in nanomedicine.[122]

A therapeutical strategy based on drug/gene co-delivery

Innovative strategies in cancer treatments can combine conventional approaches, as radiotherapy and chemotherapy, with the emerging gene therapy modality improving clinical achievements.[123,124] Following this concept, we created a pDNA based system for the sustainable delivery of anticancer drugs and genes to cancer cells. The tumor suppressor gene p53 was efficiently encapsulated into biocompatible microgels by an inverse microemulsion polymerization method using ethylene glycol diglycidyl ether (EGDE) as cross-linker.[64] (Figure 6) For 0.1% EGDE microgels, the diameter of spheres range from 0.5 to 2 μm; by increasing the cross-linker concentration, the size of spheres range from 1 to 5 μm, showing a higher heterogenous size distribution.[64] High p53 encapsulation efficiencies were obtained. Moreover, it was demonstrated that the microgel structure protect the encapsulated pDNA against serum nucleases, which is an important issue affecting both pDNA stability and transfection efficiency.

Doxorubicin (DOX) has been incorporated into pDNA gels by imbibition and, along with p53 gene, can be loaded and release from microgels; quite relevant is also the less toxic effect of the incorporated drug, as compared with naked DOX. Photodisruption of microgels can be used as a strategy to enhance release. Through a quantitative analysis using appropriate release models we found that the pDNA release follows a Super-Case II transport mechanism, while the doxorubicin is Fickian when the microgels were light irradiated.[64] Furthermore it is worth noticing that the mean dissolution time[125] of DOX, from irradiated pDNA microgels, is around 50 hours. Before being used in transfection studies, the pDNA microgels were irradiated with light (400 nm), in order to promote the microgel photodisruption and consequent release of pDNA. The p53 protein content has been determined by using the p53 pan-Elisa kit. This assay for the quantification of wild-type and mutant p53 is based on a quantitative sandwich Elisa principle. Figure 7 presents the relative quantification of p53 protein expression in cancer HeLa cells transfected with 0.1% and 0.2% EGDE pDNA microgels, as a function of initial pDNA loading amount. We stated that 0.1% EGDE microgels are able to surpass the cellular barrier and release bioactive pDNA into the cytosol. The barrier of the nucleus is permeable to foreign pDNA only during cell division; the high rate of tumour cell division, probably, enhances transfection and gene expression. Contrary, for the most cross-linked pDNA vector the success of transfection is very limited since the

quantified p53 density is not significant. For this situation, contributes the mean diameter of 0.2% pDNA microgels which is too large for efficient intracellular uptake and internalization.[64] It becomes clear the relevance of vector properties such as, size, cross-linker density and pDNA loading in the success of transfection process, of which, size seems to be the most important of all. Thus, the less cross-linked microgel carrier is more suitable for gene delivery applications since it demonstrates a good biological functionality for pDNA loading and *in vitro* gene expression. Additionally, a study using YOYO labelled p53 microgels illustrates the advantage of p53 encapsulation, as well as, the cell uptake/transfection efficiency of this pDNA carrier.[126]

Cancer cells are usually quite resistant to apoptosis and the mechanisms underlying this resistance still remain, in most of the cases, unclear. The mitochondrial activity assay, MTT, is one of the few tools used to monitor apoptosis that provides quantitative rather than qualitative results. To evaluate the inhibition of cell viability and induced cell apoptosis by the pDNA studied systems, MTT assay was applied to Hela cells treated with naked pDNA, 0.1% EGDE pDNA-lipofectamine microgel or 0.1% EGDE pDNA/DOX-lipofectamine microgel (Figure 8).[126] It is quite evident, from our studies, that the application of naked pDNA is not successful in grow inhibition or death of Hela cells. When cancer cells are exposed to p53 encoding plasmid DNA microgels a cell viability inhibition effect is observed; viable cells gradually decrease with transfection time, with a major lowering of 23% from day 4 to 6. This clearly indicates the expression of the p53 gene and its regulatory role associated with damaged DNA; the introduction of p53 into tumor cells leads to cell apoptosis. Compared with the treatments of p53 microgel and free drug separately, the simultaneous treatment by p53 and DOX with the pDNA/drug microgel has a stronger effect in reducing cell viability. Co-delivery of gene and drug to the same cells results in efficient cell inhibition and larger apoptosis, and thus p53/DOX microgels are able to effectively mediate gene transfection and drug release enhancing curing effect. The dual delivery vehicle exhibits lower percentage of viable Hela cells, illustrating the achievement of clear synergistic effect in suppressing the proliferation of tumor cells when compared to individual treatments of encapsulated p53 and pure DOX.[126]

Conclusions and future trends

In this review, we have summarized the progresses achieved by our research team in the biomedical field by using plasmid DNA based systems. This is our contribution to the advance in engineering cross-linked networks with suitable intrinsic properties for studying plasmid DNA/co-solutes interaction, in general, and for the dual delivery of genes and drugs. These pDNA gel carriers can found interesting applications as biomedical devices in a variety of clinical purposes. Although there has been a lot of significant work developed, in the last decades, aiming the targeted and controlled release of plasmid DNA there are still many issues to address, namely target the delivery of these vehicles to specific organs, tissues and cells. The future of colloidal research holds for the development of novel innovative matrices with controlling mechanical viscoelastic properties, versatile swelling performance, size and internal organization, triggered degradation behaviour, as well as for enhancing their biological interactions with body components to design and tailor appropriate devices to be used therapeutically. Furthermore, a continuous search for understanding the role of amphiphilic compounds, salts, proteins and polyelectrolytes in gel swelling behaviour is imperative to enhance network functionality, in order to design hydrogel delivery systems with control/targeted release profiles. This will, certainly, increase the efficacy within current gene therapy trials and it may also be extended to other areas such as drug delivery, scaffolds production and tissue engineering. Additionally, the efforts in molecular-scale design and in theoretical modelling will, certainly, make the use of hydrogels in the biomedical field more feasible and promising.

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Figure Captions

Figure 1. Picture of plasmid DNA gels cross-linked with 0.2% EGDE.

Figure 2. Fluorescence micrograph of a plasmid DNA gel (0.2% cross-linker), exhibiting a double stranded conformation (A) and scanning electron micrograph of a freeze-dried plasmid DNA gel (0.2% cross-linker) (B).

Figure 3. Swelling isotherms (V/V_0) for plasmid DNA gels (0.2% cross-linker) immersed in solutions of the cationic surfactants $C_{16}TAB$, $C_{14}TAB$, $C_{12}TAB$ and C_8TAB. Temperature 25ºC, pH 8.5. Adapted from Reference 86.

Figure 4. Cumulative release of pDNA from cross-linked pDNA gels with 0.2 % (■) and 0.5 % (▲) (w/v) EGDE, as a function of time. Studies were performed after the irradiation of gels with light (400 nm) (A) and in the dark conditions (B). Adapted from Reference 88.

Figure 5. Cumulative release of lysozyme, BSA and FITC-dextran from 0.2 % EGDE and 0.5 % EGDE plasmid DNA gels as a function of time, and as a function of 0.2 % EGDE gel mesh size when gels were irradiated with light (400 nm). Solid lines correspond to the best fits of experimental data to equation (2). Adapted from Reference 88.

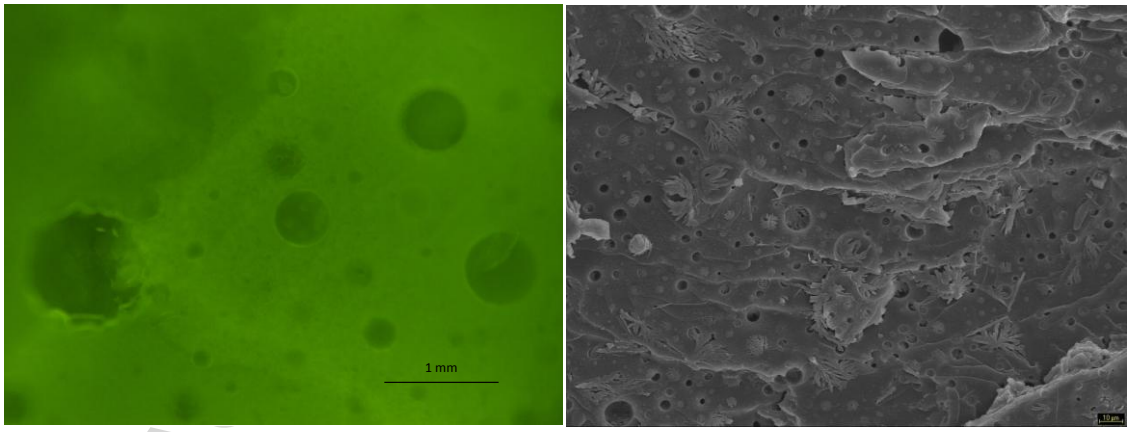
Figure 6. Scanning electron micrograph of plasmid DNA microgels cross-linked with 0.1% (w/v) EGDE (A) and 0.2% (w/v) EGDE (B). Adapted from Reference 64.

Figure 7. Quantification of p53 protein expression in cancer HeLa cells using a pan-p53 Elisa kit, for 0.1 % and 0.2 % (w/v) EGDE cross-linked pDNA microgels (4 or 6 μg/ml pDNA loading). The data were obtained by calculating the average of 3 independent experiments. The respective errors were determined and were below 0.05 %. Adapted from Reference 126.

Figure 8. Viability of Hela cells after transfection with naked pDNA (6.0 μg/ml) (A), 0.1% (w/v) EGDE pDNA-lipofectamine microgel (B) or 0.1% (w/v) EGDE pDNA/DOX-lipofectamine microgel (C) after 1, 2, 4, 6 and 8 days measured by MTT assay. Percent viability is expressed relative to control cells. The data were obtained by calculating the average of 3 experiments. The respective errors were determined and were below 0.05%. Adapted from Reference 126.

Figures

Figure 1.



A B

Figure 2.

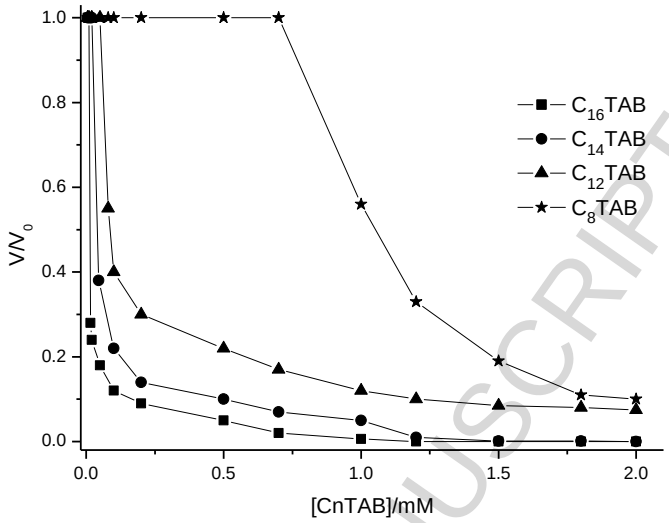
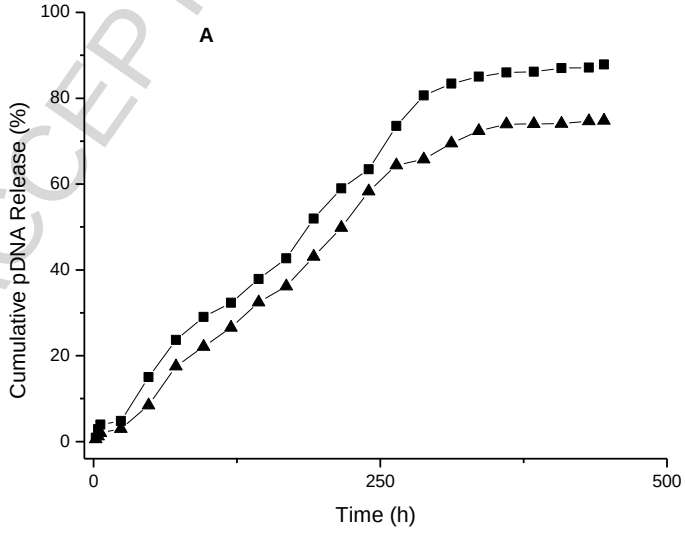


Figure 3.



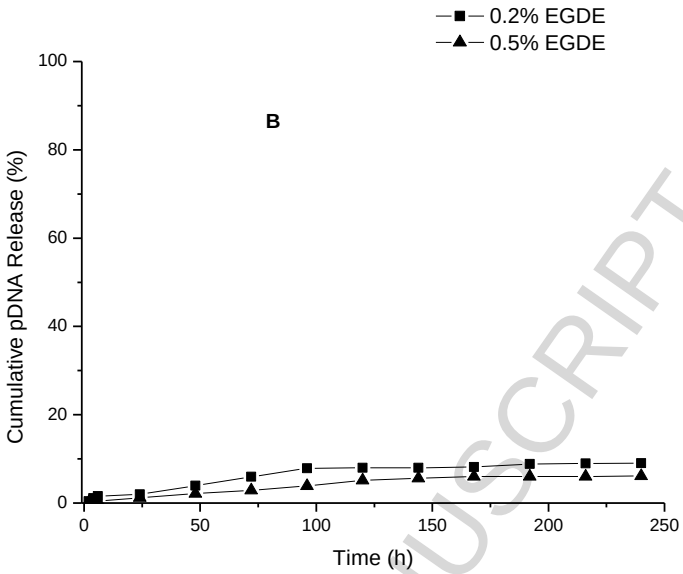


Figure 4.

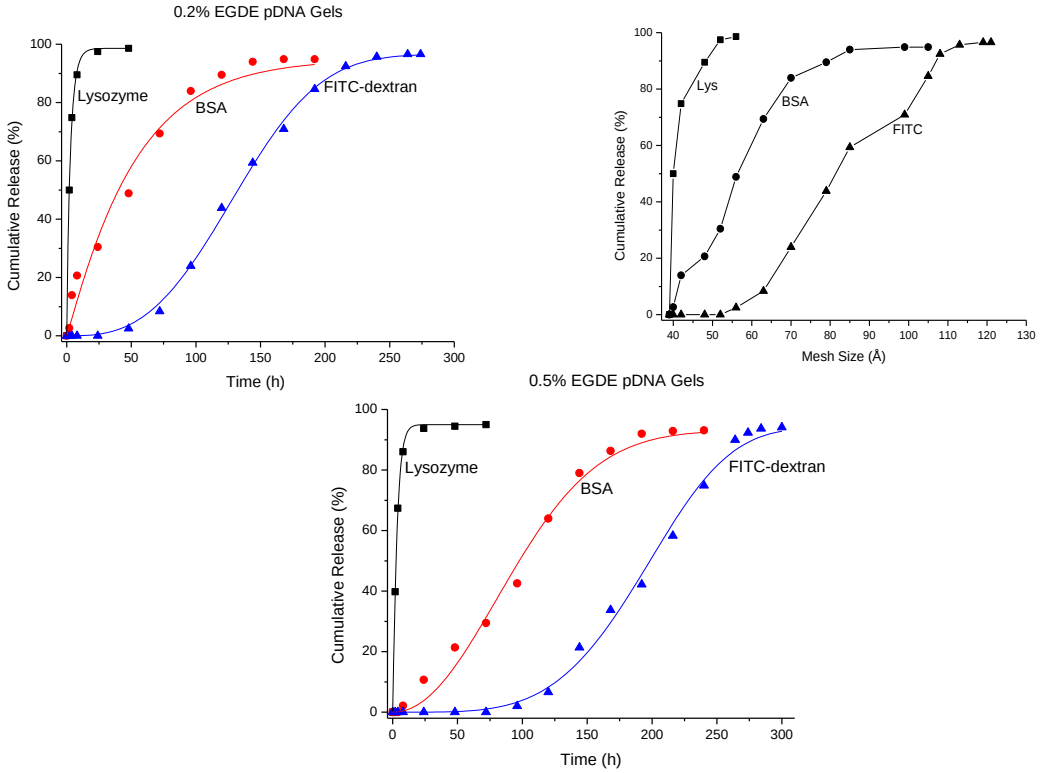
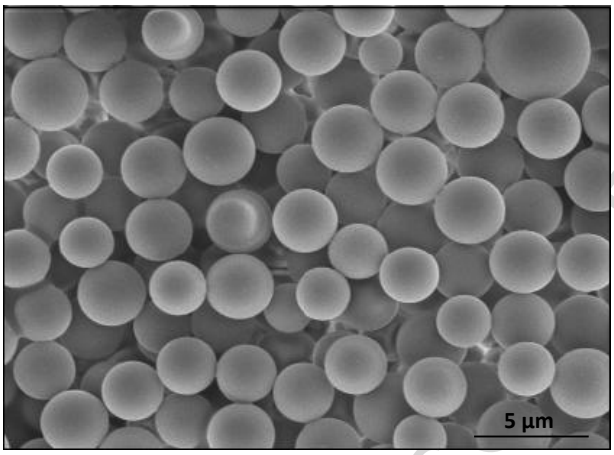
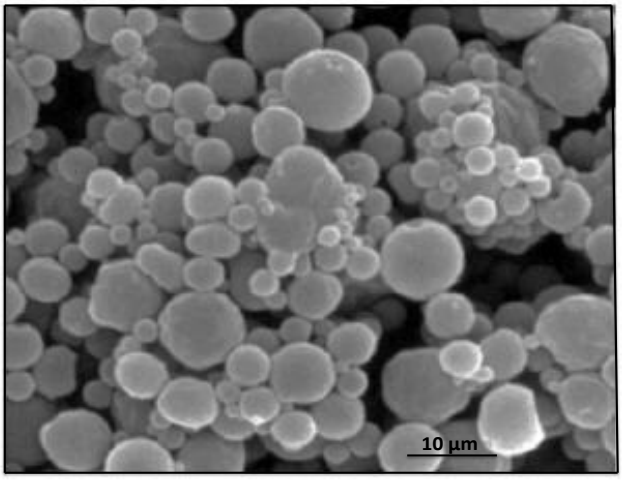


Figure 5.



A



B

Figure 6.

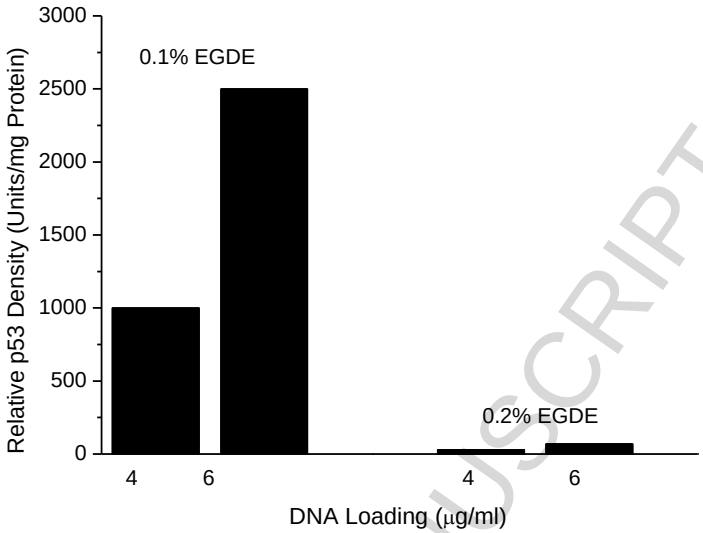
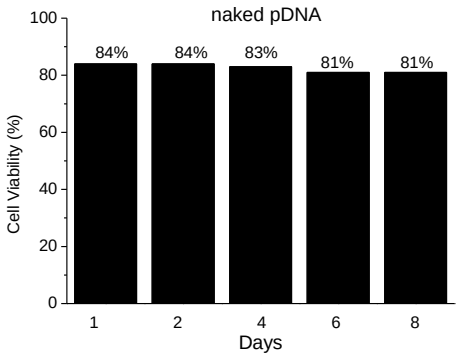
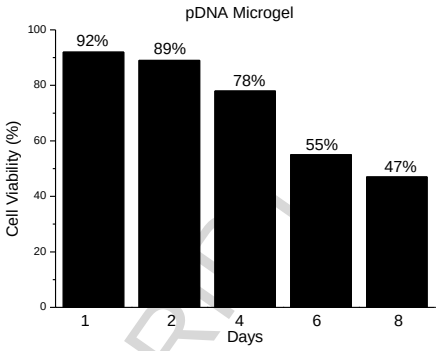


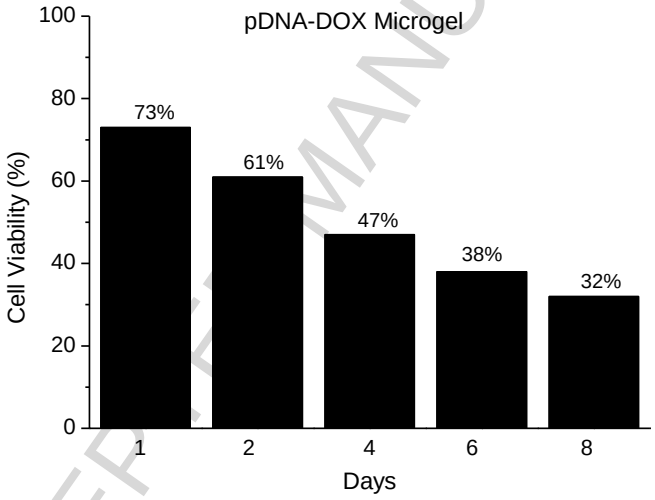
Figure 7.



A



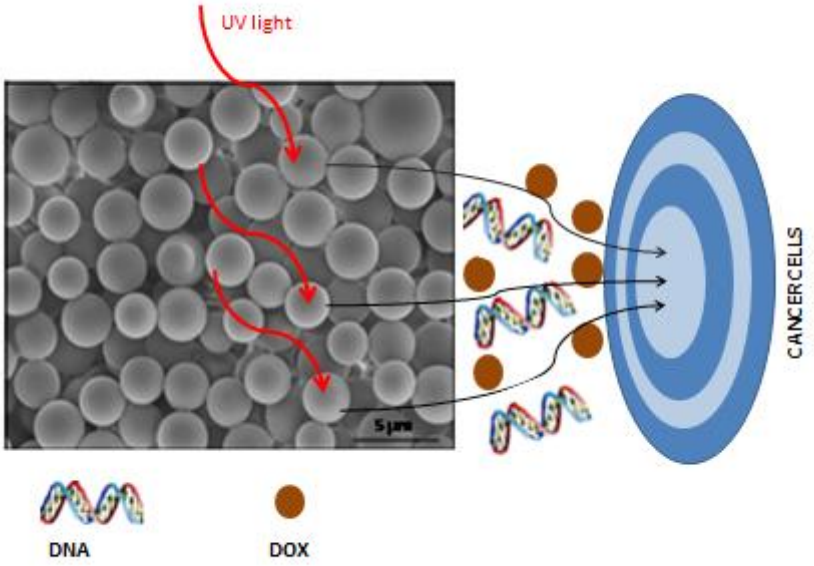
B



C

Figure 8.

Graphical Abstract



Highlights

· Biocompatible macro and micro plasmid DNA gels were prepared by a cross-linking reaction for biomedical use.

· For the first time, the pDNA gels have been investigated with respect to their swelling behaviour in the present of additives; from this, the nature of the interactions can be inferred.

· The pDNA based carriers can simultaneously load and release genes and anticancer drugs, upon photodegradation, in a controlled and sustained manner.

· The combination of chemical and gene therapies has a strong effect in cancer cell apoptosis and tumour suppression.

· Our cancer therapy approach will inspire researchers to design and develop efficient systems for drug/gene co-delivery aiming the challenging cancer cure.